

Hematophagy of *Lutzomyia longipalpis* (Diptera; Psychodidae) on a mouse skin model: a new view based on intravital microscopy and image analysis

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The sand fly *Lutzomyia longipalpis* is considered as the main vector of *Leishmania infantum*, the causative agent of Visceral Leishmaniasis in the Americas. Despite its medical importance, various aspects of feeding behaviour of sand flies are still not clear. In this work the saliva of sand flies was labelled with 0.1% Acridine Orange in saturated sucrose and the softwares Image J and Matlab were used for the image and signal analyses. We found that during probing phase the saliva was released shortly after each bite. Surprisingly, during ingestion of blood by females the continuous squirts of saliva were observed inside blood vessels, and saliva has been often carried by the flow (n=17/54). Instead of feeding from capillaries sand flies were feeding primarily from the flow of arterioles and venules. During the feeding process the pattern of blood ingestion consisted of large peaks with low frequency (0.27 Hz) and small oscillations with high frequency (3.7 Hz , n=8). The vascular network of mice experienced several changes during blood ingestion: large accumulation of platelets, vasodilatation and leukocytes recruitment, including the observation of the ingestion of leukocytes by the insects. In this study we describe the blood feeding process of the sand fly *L. longipalpis* showing for the first time its salivation pattern and its effect on mouse skin using different techniques of intravital microscopy and image analysis.